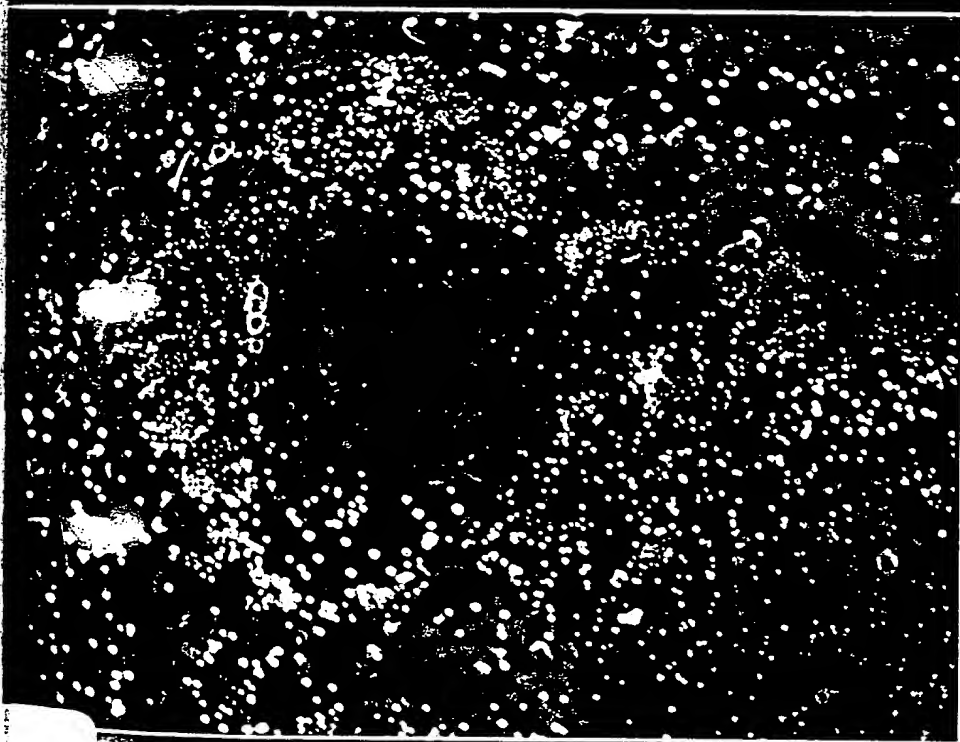


The Journal of Clinical Investigation

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■ molecular medicine / genetic disorders

■ infection / inflammation / immunity

■ hormones / cytokines / signaling

■ cell growth & differentiation

■ cellular, transport & organ physiology

■ atherosclerosis / thrombosis / metabolism

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Exhibit 29

The Journal of Clinical Investigation
October 1991, Volume 88, Number 4

- * In search of Mr. Wizard. Presidential address to the American Society for Clinical Investigation, Seattle, Washington, 4 May 1991. *W. J. Koopman* 1063

■ **Molecular Medicine / Genetic Disorders**

- Illegitimate transcription. Application to the analysis of truncated transcripts of the dystrophin gene in nonmuscle cultured cells from Duchenne and Becker patients. *J. Chelly, H. Gilgenkrantz, J. P. Hugnot, G. Hamard, M. Lambert, D. Récan, S. Akli, M. Cometto, A. Kahn, and J. C. Kaplan* 1161

- Deficiency of skeletal muscle succinate dehydrogenase and aconitase. Pathophysiology of exercise in a novel human muscle oxidative defect. *R. G. Haller, K. G. Henriksson, L. Jorfeldt, E. Hultman, R. Wibom, K. Sahlin, N.-H. Areskog, M. Gunder, K. Ayyad, C. G. Blomqvist, R. E. Hall, P. Thuillier, N. G. Kennaway, and S. F. Lewis* 1197

- Analysis of the gene sequences of the insulin receptor and the insulin-sensitive glucose transporter (GLUT-4) in patients with common-type non-insulin-dependent diabetes mellitus. *J. Kusari, U. S. Verma, J. B. Buse, R. R. Henry, and J. M. Olefsky* 1323

- * An in vivo animal model of gene therapy for leukocyte adhesion deficiency. *J. C. Krauss, L. A. Mayo-Bond, C. E. Rogers, K. L. Weber, R. F. Todd III, and J. M. Wilson* 1412

- * Identification and regulation of the cystic fibrosis transmembrane conductance regulator-generated chloride channel. *H. A. Berger, M. P. Anderson, R. J. Gregory, S. Thompson, P. W. Howard, R. A. Maurer, R. Mulligan, A. E. Smith, and M. J. Welsh* 1422

■ **Infection / Inflammation / Immunity**

- Killing of gram-negative bacteria by lactoferrin and lysozyme. *R. T. Ellison III and T. J. Giehl* 1080

- Pseudomonas* and neutrophil products modify transferrin and lactoferrin to create conditions that favor hydroxyl radical formation. *B. E. Britigan and B. L. Edeker* 1092

- Lactoferrin inhibits or promotes *Legionella pneumophila* intracellular multiplication in nonactivated and interferon gamma-activated human monocytes depending upon its degree of iron saturation. Iron-lactoferrin and nonphysiologic iron chelates reverse monocyte activation against *Legionella pneumophila*. *T. F. Byrd and M. A. Horwitz* 1103

- An activated CD8+ lymphocyte appears in lymph nodes of rhesus monkeys early after infection with simian immunodeficiency virus. *K. A. Reimann, G. B. Snyder, L. V. Chalifoux, B. C. D. Waite, M. D. Miller, H. Yamamoto, O. Spertini, and N. L. Letvin* 1113

- Adherence of neutrophils to canine cardiac myocytes in vitro is dependent on intercellular adhesion molecule-1. *C. Smith, M. L. Entman, C. L. Lane, A. L. Beaudet, T. I. Ty, K. Youker, H. K. Hawkins, and D. C. Anderson* 1216

- Staphylococci surviving intracellularly in phagocytes from patients suffering from chronic granulomatous disease are killed in vitro by antibiotics encapsulated in liposomes. *J. Roesler, S. Hockertz, B. Vogt, and M.-L. Lohmann-Matthes* 1224

- Requirement of CD4-positive T cells for cellular recruitment to the lungs of mice in response to a particulate intratracheal antigen. *J. L. Curtis, P. K. Byrd, M. L. Warnock, and H. B. Kalireider* 1244

- Detection of human T cell lymphotropic virus type I proviral DNA and its gene expression in synovial cells in chronic inflammatory arthropathy. *I. Kitajima, K. Yamamoto, K. Sato, Y. Nakajima, T. Nakajima, I. Maruyama, M. Osame, and K. Nishioka* 1315

- Immunosuppressive activity of 13-*cis*-retinoic acid and prevention of experimental autoimmune encephalomyelitis in rats. *L. Massacesi, E. Castigli, M. Vergelli, J. Olivetto, A. L. Abbamondi, F. Sarlo, and L. Amaducci* 1331

- LKM-1 autoantibodies recognize a short linear sequence in P450IID6, a cytochrome P-450 monooxygenase. *M. P. Manns, K. J. Griffin, K. F. Sullivan, and E. F. Johnson* 1370

- Identification and partial characterization of angiogenesis bioactivity in the lower respiratory tract after acute lung injury. *C. Henke, V. Fiegel, M. Peterson, M. Wick, D. Knighton, J. McCarthy, and P. Bitterman* 1386

- Role of endothelial-leukocyte adhesion molecule 1 (ELAM-1) in neutrophil-mediated lung injury in rats. *M. S. Mulligan, J. Varani, M. K. Dame, C. L. Lane, C. W. Smith, D. C. Anderson, and P. A. Ward* 1396

- * Endothelial leukocyte adhesion molecule-1 mediates antigen-induced acute airway inflammation and late-phase airway obstruction in monkeys. *R. H. Gundel, C. D. Wegner, C. A. Torcellini, C. C. Clarke, N. Haynes, R. Rothlein, C. W. Smith, and L. G. Letts* 1407

- * Interleukin-5 and the posttreatment eosinophilia in patients with onchocerciasis. *A. P. Limaye, J. S. Abrams, J. E. Silver, K. Awadzi, H. F. Francis, E. A. Ottesen, and T. B. Nutman* 1418

■ **Hormones / Cytokines / Signaling**

- Endothelial cells modulate renin secretion from isolated mouse juxtaglomerular cells. *A. Kurtz, B. Kaissling, R. Busse, and W. Baier* 1147

- Oligonucleotides antisense to the interleukin 1 receptor mRNA block the effects of interleukin 1 in cultured murine and human fibroblasts and in mice. *R. M. Burch and L. C. Mahan* 1190

- Evidence for entry of plasma insulin into cerebrospinal fluid through an intermediate compartment in dogs. Quantitative aspects and implications for transport. *M. W. Schwartz, R. N. Bergman, S. E. Kahn, G. J. Tabor, Jr., L. D. Fisher, A. J. Sipols, S. C. Woods, G. M. Steil, and D. Porte, Jr.* 1272

(continued on the reverse side of this cover)

JCINAO 88(4) 1063-1434 (1991)

Role of L-thyroxine in nuclear thyroid hormone receptor occupancy and growth hormone production in cultured GC cells. <i>Y. Halperin, L. E. Shapiro, and M. I. Surks</i>	1291	Effects of cocaine on epicardial coronary artery reactivity in miniature swine after endothelial injury and high cholesterol feeding. In vivo and in vitro analysis. <i>K. Egashira, F. S. Pipers, and J. P. Morgan</i>	1307
A unique receptor-independent mechanism by which insulinlike growth factor I regulates the availability of insulinlike growth factor binding proteins in normal and transformed human fibroblasts. <i>C. A. Conover</i>	1354	Sphingolipids are required for mammalian epidermal barrier function. Inhibition of sphingolipid synthesis delays barrier recovery after acute perturbation. <i>W. M. Holleran, M. Mao-Qiang, W. N. Gao, G. K. Menon, P. M. Elias, and K. R. Feingold</i>	1338
Red blood cells are a sink for interleukin 8, a leukocyte chemotaxin. <i>W. C. Darbonne, G. C. Rice, M. A. Mohler, T. Apple, C. A. Hébert, A. J. Valente, and J. B. Baker</i>	1362	Sodium uptake across basolateral membrane of rat distal colon. Evidence for Na-H exchange and Na-anion cotransport. <i>V. M. Rajendran, M. Oesterlin, and H. J. Binder</i>	1379
■ Cell Growth and Differentiation		■ Atherosclerosis / Thrombosis / Metabolism	
* The plasminogen activator/plasmin system. <i>J.-D. Vassalli, A.-P. Sappino, and D. Belin</i>	1067	Monocyte chemoattractant protein-1 in human atheromatous plaques. <i>N. A. Nelken, S. R. Coughlin, D. Gordon, and J. N. Wilcox</i>	1121
Differential protease expression by cutaneous squamous and basal cell carcinomas. <i>A.-P. Sappino, D. Belin, J. Huarte, S. Hirschel-Scholz, J.-H. Saurat, and J.-D. Vassalli</i>	1073	Ligand bridging mediates integrin $\alpha_{IIb}\beta_3$ (platelet GPIIb-IIIa) dependent homotypic and heterotypic cell-cell interactions. <i>M. P. Gawaz, J. C. Loftus, M. L. Bajt, M. M. Frojmovic, E. F. Plow, and M. H. Ginsberg</i>	1128
Decreased DNA synthesis by cultured osteoblastic cells in eugonadal osteoporotic men with defective bone formation. <i>P. J. Marie, M. C. de Vernejoul, D. Connes, and M. Hott</i>	1167	Reduction of contact activation related fibrinolytic activity in factor XII deficient patients. Further evidence for the role of the contact system in fibrinolysis in vivo. <i>M. Levi, C. E. Hack, J. P. de Boer, D. P. M. Brandjes, H. R. Büller, and J. Wouter ten Cate</i>	1155
■ Cellular, Transport and Organ Physiology		Use of an anti-low density lipoprotein receptor antibody to quantify the role of the LDL receptor in the removal of chylomicron remnants in the mouse in vivo. <i>S. Y. Choi, L. G. Fong, M. J. Kirven, and A. D. Cooper</i>	1173
A pH modifier site regulates activity of the $\text{Na}^+:\text{HCO}_3^-$ cotransporter in basolateral membranes of kidney proximal tubules. <i>M. Soleimani, G. A. Lesoine, J. A. Bergman, and T. D. McKinney</i>	1135	Effect of the antilipolytic nicotinic acid analogue acipimox on whole-body and skeletal muscle glucose metabolism in patients with non-insulin-dependent diabetes mellitus. <i>A. Vaag, P. Skött, P. Damsbo, M.-A. Gall, E. A. Richter, and H. Beck-Nielsen</i>	1282
Alteration of collagen phenotypes in ischemic cardiomyopathy. <i>D. Mukherjee and S. Sen</i>	1141	Lipoprotein lipase modulates net secretory output of apolipoprotein B in vitro. A possible pathophysiologic explanation for familial combined hyperlipidemia. <i>K. J. Williams, K. A. Petrie, R. W. Brocia, and T. L. Swenson</i>	1300
Fibronectin biosynthesis in the rat aorta in vitro. Changes due to experimental hypertension. <i>R. Saouaf, I. Takasaki, E. Eastman, A. V. Chobanian, and P. Brecher</i>	1182	Regulation of murine type I plasminogen activator inhibitor gene expression in vivo. Tissue specificity and induction by lipopolysaccharide, tumor necrosis factor- α , and transforming growth factor- β . <i>M. S. Sawdey and D. J. Loskutoff</i>	1346
Alterations in the structure, physicochemical properties, and pH of hepatocyte lysosomes in experimental iron overload. <i>B. M. Myers, F. G. Prendergast, R. Holman, S. M. Kuntz, and N. F. LaRusso</i>	1207	October Author Index	1432
Insulin attenuates vasopressin-induced calcium transients and a voltage-dependent calcium response in rat vascular smooth muscle cells. <i>P. R. Standley, F. Zhang, J. L. Ram, M. B. Zemel, and J. R. Sowers</i>	1230	Correction	1433
Antibody to CD-18 exerts endothelial and cardiac protective effects in myocardial ischemia and reperfusion. <i>X.-L. Ma, P. S. Tsao, and A. M. Lefer</i>	1237		
Intracellular Mg^{2+} and magnesium depletion in isolated renal thick ascending limb cells. <i>L.-J. Dai and G. A. Quamme</i>	1255		
Glycochenodeoxycholic acid inhibits calcium phosphate precipitation in vitro by preventing the transformation of amorphous calcium phosphate to calcium hydroxyapatite. <i>S.-M. Qiu, G. Wen, N. Hirakawa, R. D. Soloway, N.-K. Hong, and R. S. Crowther</i>	1265		

* Perspectives article
† Rapid Publication

Cover picture: Localization of urokinase-type plasminogen activator mRNA in tumor cells surrounding a horn pearl in a cutaneous squamous cell carcinoma. See related article in this issue by Sappino et al., pp. 1073-1079.

Evidence for entry of plasma insulin into cerebrospinal fluid through an intermediate compartment in dogs. Quantitative aspects and implications for transport.

M. W. Schwartz, R. N. Bergman, S. E. Kahn, G. J. Taborsky, Jr., L. D. Fisher, A. J. Sipols, S. C. Woods, G. M. Steil, and D. Porte, Jr. 1272

Effect of the antilipolytic nicotinic acid analogue acipimox on whole-body and skeletal muscle glucose metabolism in patients with non-insulin-dependent diabetes mellitus.

A. Vaag, P. Sködt, P. Damsbo, M.-A. Gall, E. A. Richter, and H. Beck-Nielsen 1282

Role of L-thyroxine in nuclear thyroid hormone receptor occupancy and growth hormone production in cultured GC cells.

Y. Halperin, L. E. Shapiro, and M. I. Surks 1291

Lipoprotein lipase modulates net secretory output of apolipoprotein B in vitro. A possible pathophysiologic explanation for familial combined hyperlipidemia.

K. J. Williams, K. A. Petrie, R. W. Brocia, and T. L. Swenson 1300

Effects of cocaine on epicardial coronary artery reactivity in miniature swine after endothelial injury and high cholesterol feeding. In vivo and in vitro analysis.

K. Egashira, F. S. Pipers, and J. P. Morgan 1307

Detection of human T cell lymphotropic virus type I proviral DNA and its gene expression in synovial cells in chronic inflammatory arthropathy.

I. Kitajima, K. Yamamoto, K. Sato, Y. Nakajima, T. Nakajima, I. Maruyama, M. Osame, and K. Nishioka 1315

Analysis of the gene sequences of the insulin receptor and the insulin-sensitive glucose transporter (GLUT-4) in patients with common-type non-insulin-dependent diabetes mellitus.

J. Kusari, U. S. Verma, J. B. Buse, R. R. Henry, and J. M. Olefsky 1323

Immunosuppressive activity of 13-*cis*-retinoic acid and prevention of experimental autoimmune encephalomyelitis in rats.

L. Massacesi, E. Castigli, M. Vergelli, J. Olivetto, A. L. Abbamondi, F. Sarlo, and L. Amaducci 1331

Sphingolipids are required for mammalian epidermal barrier function. Inhibition of sphingolipid synthesis delays barrier recovery after acute perturbation.

W. M. Holleran, M. Mao-Qiang, W. N. Gao, G. K. Menon, P. M. Elias, and K. R. Feingold 1338

Regulation of murine type 1 plasminogen activator inhibitor gene expression in vivo. Tissue specificity and induction by lipopolysaccharide, tumor necrosis factor- α , and transforming growth factor- β .

M. S. Sawdey and D. J. Loskutoff 1346

A unique receptor-independent mechanism by which insulinlike growth factor I regulates the availability of insulinlike growth factor binding proteins in normal and transformed human fibroblasts.

C. A. Conover 1354

Red blood cells are a sink for interleukin 8, a leukocyte chemotaxin.

W. C. Darbonne, G. C. Rice, M. A. Mohler, T. Apple, C. A. Hébert, A. J. Valente, and J. B. Baker 1362

LKM-1 autoantibodies recognize a short linear sequence in P450IID6, a cytochrome P-450 monooxygenase.

M. P. Manns, K. J. Griffin, K. F. Sullivan, and E. F. Johnson 1370

Sodium uptake across basolateral membrane of rat distal colon. Evidence for Na-H exchange and Na-anion cotransport.

V. M. Rajendran, M. Oesterlin, and H. J. Binder 1379

Identification and partial characterization of angiogenesis bioactivity in the lower respiratory tract after acute lung injury.

C. Henke, V. Fiegel, M. Peterson, M. Wick, D. Knighton, J. McCarthy, and P. Bitterman 1386

Role of endothelial-leukocyte adhesion molecule 1 (ELAM-1) in neutrophil-mediated lung injury in rats.

M. S. Mulligan, J. Varani, M. K. Dame, C. L. Lane, C. W. Smith, D. C. Anderson, and P. A. Ward 1396

Rapid Publications

Endothelial leukocyte adhesion molecule-1 mediates antigen-induced acute airway inflammation and late-phase airway obstruction in monkeys.

R. H. Gundel, C. D. Wegner, C. A. Torcellini, C. C. Clarke, N. Haynes, R. Rothlein, C. W. Smith, and L. G. Letts 1407

An in vivo animal model of gene therapy for leukocyte adhesion deficiency.

J. C. Krauss, L. A. Mayo-Bond, C. E. Rogers, K. L. Weber, R. F. Todd III, and J. M. Wilson 1412

Interleukin-5 and the posttreatment eosinophilia in patients with onchocerciasis.

A. P. Limaye, J. S. Abrams, J. E. Silver, K. Awadzi, H. F. Francis, E. A. Ottesen, and T. B. Nutman 1418

Identification and regulation of the cystic fibrosis transmembrane conductance regulator-generated chloride channel.

H. A. Berger, M. P. Anderson, R. J. Gregory, S. Thompson, P. W. Howard, R. A. Maurer, R. Mulligan, A. E. Smith, and M. J. Welsh 1422

October Author Index 1432

Correction 1433

The Journal of Clinical Investigation

October 1991, Volume 88, Number 4

Perspectives

- In search of Mr. Wizard. Presidential address to the American Society for Clinical Investigation, Seattle, Washington, 4 May 1991. *W. J. Koopman* 1063

- The plasminogen activator/plasmin system. *J.-D. Vassalli, A.-P. Sappino, and D. Belin* 1067

Regular Articles

- Differential protease expression by cutaneous squamous and basal cell carcinomas. *A.-P. Sappino, D. Belin, J. Huarte, S. Hirschel-Scholz, J.-H. Saurat, and J.-D. Vassalli* 1073
- Killing of gram-negative bacteria by lactoferrin and lysozyme. *R. T. Ellison III and T. J. Giehl* 1080
- Pseudomonas* and neutrophil products modify transferrin and lactoferrin to create conditions that favor hydroxyl radical formation. *B. E. Britigan and B. L. Edeker* 1092
- Lactoferrin inhibits or promotes *Legionella pneumophila* intracellular multiplication in nonactivated and interferon gamma-activated human monocytes depending upon its degree of iron saturation. Iron-lactoferrin and nonphysiologic iron chelates reverse monocyte activation against *Legionella pneumophila*. *T. F. Byrd and M. A. Horwitz* 1103
- An activated CD8+ lymphocyte appears in lymph nodes of rhesus monkeys early after infection with simian immunodeficiency virus. *K. A. Reimann, G. B. Snyder, L. V. Chalifoux, B. C. D. Waite, M. D. Miller, H. Yamamoto, O. Spertini, and N. L. Letvin* 1113
- Monocyte chemoattractant protein-1 in human atherosclerotic plaques. *N. A. Nelken, S. R. Coughlin, D. Gordon, and J. N. Wilcox* 1121
- Ligand bridging mediates integrin $\alpha_{IIb}\beta_3$ (platelet GPIIb-IIIa) dependent homotypic and heterotypic cell-cell interactions. *M. P. Gawaz, J. C. Loftus, M. L. Bajt, M. M. Frojmovic, E. F. Plow, and M. H. Ginsberg* 1128
- A pH modifier site regulates activity of the $\text{Na}^+/\text{HCO}_3^-$ cotransporter in basolateral membranes of kidney proximal tubules. *M. Soleimani, G. A. Lesoine, J. A. Bergman, and T. D. McKinney* 1135
- Alteration of collagen phenotypes in ischemic cardiomyopathy. *D. Mukherjee and S. Sen* 1141
- Endothelial cells modulate renin secretion from isolated mouse juxtaglomerular cells. *A. Kurtz, B. Kaissling, R. Busse, and W. Baier* 1147
- Reduction of contact activation related fibrinolytic activity in factor XII deficient patients. Further evidence for the role of the contact system in fibrinolysis in vivo. *M. Levi, C. E. Hack, J. P. de Boer, D. P. M. Brandjes, H. R. Büller, and J. Wouter ten Cate* 1155
- Illegitimate transcription. Application to the analysis of truncated transcripts of the dystrophin gene in nonmuscle cultured cells from Duchenne and Becker patients. *J. Chelly, H. Gilgenkrantz, J. P. Hugnot, G. Hamard, M. Lambert, D. Récan, S. Akli, M. Cometto, A. Kahn, and J. C. Kaplan* 1161
- Decreased DNA synthesis by cultured osteoblastic cells in eugonadal osteoporotic men with defective bone formation. *P. J. Marie, M. C. de Vernejoul, D. Connes, and M. Hott* 1167
- Use of an anti-low density lipoprotein receptor antibody to quantify the role of the LDL receptor in the removal of chylomicron remnants in the mouse in vivo. *S. Y. Choi, L. G. Fong, M. J. Kirven, and A. D. Cooper* 1173
- Fibronectin biosynthesis in the rat aorta in vitro. Changes due to experimental hypertension. *R. Saouaf, I. Takasaki, E. Eastman, A. V. Chobanian, and P. Brecher* 1182
- Oligonucleotides antisense to the interleukin 1 receptor mRNA block the effects of interleukin 1 in cultured murine and human fibroblasts and in mice. *R. M. Burch and L. C. Mahan* 1190
- Deficiency of skeletal muscle succinate dehydrogenase and aconitase. Pathophysiology of exercise in a novel human muscle oxidative defect. *R. G. Haller, K. G. Henriksson, L. Jorfeldt, E. Hultman, R. Wiborn, K. Sahlin, N.-H. Areskog, M. Gunder, K. Ayyad, C. G. Blomqvist, R. E. Hall, P. Thuillier, N. G. Kennaway, and S. F. Lewis* 1197
- Alterations in the structure, physicochemical properties, and pH of hepatocyte lysosomes in experimental iron overload. *B. M. Myers, F. G. Prendergast, R. Holman, S. M. Kuntz, and N. F. LaRusso* 1207
- Adherence of neutrophils to canine cardiac myocytes in vitro is dependent on intercellular adhesion molecule-1. *C. W. Smith, M. L. Entman, C. L. Lane, A. L. Beaudet, T. I. Ty, K. Youker, H. K. Hawkins, and D. C. Anderson* 1216
- Staphylococci surviving intracellularly in phagocytes from patients suffering from chronic granulomatous disease are killed in vitro by antibiotics encapsulated in liposomes. *J. Roesler, S. Hockertz, B. Vogt, and M.-L. Lohmann-Matthes* 1224
- Insulin attenuates vasopressin-induced calcium transients and a voltage-dependent calcium response in rat vascular smooth muscle cells. *P. R. Standley, F. Zhang, J. L. Ram, M. B. Zemel, and J. R. Sowers* 1230
- Antibody to CD-18 exerts endothelial and cardiac protective effects in myocardial ischemia and reperfusion. *X.-L. Ma, P. S. Tsao, and A. M. Lefer* 1237
- Requirement of CD4-positive T cells for cellular recruitment to the lungs of mice in response to a particulate intratracheal antigen. *J. L. Curtis, P. K. Byrd, M. L. Warnock, and H. B. Kaltreider* 1244
- Intracellular Mg^{2+} and magnesium depletion in isolated renal thick ascending limb cells. *L.-J. Dai and G. A. Quamme* 1255
- Glycochenodeoxycholic acid inhibits calcium phosphate precipitation in vitro by preventing the transformation of amorphous calcium phosphate to calcium hydroxyapatite. *S.-M. Qiu, G. Wen, N. Hirakawa, R. D. Soloway, N.-K. Hong, and R. S. Crowther* 1265

The Journal of Clinical Investigation
November 1991, Volume 88, Number 5

Perspectives

- Molecular genetics of intestinal glucose transport.
*E. M. Wright, E. Turk, B. Zabel, S. Mundlos,
and J. Dyer* 1435
- Genetic causes of aortic aneurysms. Unlearning at least
part of what the textbooks say. *H. Kuivaniemi, G. Tromp,
and D. J. Prockop* 1441
- Interleukin 1 receptor antagonist. A new member of the
interleukin 1 family. *W. P. Arend* 1445

Regular Articles

- Increased expression of the interleukin 1 receptor on
blood neutrophils of humans with the sepsis syndrome.
M. B. Fasano, S. Cousart, S. Neal, and C. E. McCall . . 1452
- Molecular cloning and characterization of recombinant
parasite antigens for immunodiagnosis of onchocerciasis.
*R. Chandrashekar, K. Masood, R. M. Alvarez,
A. F. Ogunrinade, R. Lujan, F. O. Richards, Jr.,
and G. J. Weil* 1460
- Antimalarial effects of peptide inhibitors of a *Plasmodium*
falciparum cysteine proteinase. *P. J. Rosenthal,
W. S. Wollish, J. T. Palmer, and D. Rasnick* 1467
- Activation of T lymphocytes in dengue virus infections.
High levels of soluble interleukin 2 receptor, soluble CD4,
soluble CD8, interleukin 2, and interferon- γ in sera of
children with dengue. *I. Kurane, B. L. Innis,
S. Nimmannitya, A. Nisalak, A. Meager, J. Janus,
and F. A. Ennis* 1473
- Failure of atrial natriuretic factor to increase with saline
load in patients with dilated cardiomyopathy and mild
heart failure. *M. Volpe, C. Tritto, N. De Luca, A. F. Mele,
G. Lembo, S. Rubattu, M. Romano, P. De Campora,
I. Enea, B. Ricciardelli, B. Trimarco, and M. Condorelli* 1481
- Influence of apolipoprotein E polymorphism on
apolipoprotein B-100 metabolism in normolipemic
subjects. *T. Demant, D. Bedford, C. J. Packard,
and J. Shepherd* 1490
- Feedback inhibition of cyclic adenosine monophosphate-
stimulated Na^+ transport in the rabbit cortical collecting
duct via Na^+ -dependent basolateral Ca^{++} entry.
M. D. Breyer 1502
- Hydrogen peroxide-mediated toxicity for *Leishmania*
donovani chagasi promastigotes. Role of hydroxyl radical
and protection by heat shock. *J. H. Zarley, B. E. Britigan,
and M. E. Wilson* 1511
- Mesangial cell autoantigens in immunoglobulin A
nephropathy and Henoch-Schönlein purpura.
D. J. O'Donoghue, A. Darvill, and F. W. Ballardie . . . 1522
- Escherichia coli* hemolysin is a potent inducer of
phosphoinositide hydrolysis and related metabolic
responses in human neutrophils. *F. Grimminger,
U. Sibelius, S. Bhakdi, N. Suttrop, and W. Seeger* . . . 1531
- Deficiency in phosphorylase phosphatase activity despite
elevated protein phosphatase type-1 catalytic subunit in
skeletal muscle from insulin-resistant subjects.
*B. L. Nyomba, D. L. Brautigan, K. K. Schlender,
W. Wang, C. Bogardus, and D. M. Mott* 1540

- Effect of denervation on the expression of two glucose
transporter isoforms in rat hindlimb muscle. *N. E. Block,
D. R. Menick, K. A. Robinson, and M. G. Buse* 1546
- Evidence from oocyte expression that the erythrocyte
water channel is distinct from band 3 and the glucose
transporter. *R. Zhang, S. L. Alper, B. Thorens,
and A. S. Verkman* 1553
- L-Arginine abrogates salt-sensitive hypertension in Dahl/
Rapp rats. *P. Y. Chen and P. W. Sanders* 1559
- von Willebrand factor binding to platelet GpIb initiates
signals for platelet activation. *M. H. Kroll, T. S. Harris,
J. L. Moake, R. I. Handin, and A. I. Schafer* 1568
- Regulation of transforming growth factor- β 1 gene
expression by glucocorticoids in normal human T
lymphocytes. *O. AyanlarBatuman, A. P. Ferrero, A. Diaz,
and S. A. Jimenez* 1574
- The vascular smooth muscle α -actin gene is reactivated
during cardiac hypertrophy provoked by load.
*F. M. Black, S. E. Packer, T. G. Parker, L. H. Michael,
R. Roberts, R. J. Schwartz, and M. D. Schneider* . . . 1581
- Systemic lysis protects against the effects of platelet
activation during coronary thrombolysis. *D. J. Fitzgerald,
M. Hanson, and G. A. FitzGerald* 1589
- Cytogenetic and molecular genetic studies of follicular and
papillary thyroid cancers. *M. A. Herrmann, I. D. Hay,
D. H. Bartelt, Jr., S. R. Ritland, R. J. Dahl, C. S. Grant,
and R. B. Jenkins* 1596
- Neutrophil migration across a cultured intestinal
epithelium. Dependence on a CD11b/CD18-mediated
event and enhanced efficiency in physiological direction.
C. A. Parkos, C. Delp, M. A. Arnaout, and J. L. Madara 1605
- Mithramycin inhibits SP1 binding and selectively inhibits
transcriptional activity of the dihydrofolate reductase
gene in vitro and in vivo. *S. W. Blume, R. C. Snyder,
R. Ray, S. Thomas, C. A. Koller, and D. M. Miller* . . . 1613
- Accumulation of hyaluronan and tissue edema in
experimental myocardial infarction. *A. Waldenström,
H. J. Martinussen, B. Gerdin, and R. Hällgren* 1622
- Differences in insulin action as a function of original
anatomical site of newly differentiated adipocytes
obtained in primary culture. *C. Szalryd, S. Azhar,
and G. M. Reaven* 1629
- Angiotensin inhibition potentiates the renal responses to
neutral endopeptidase inhibition in dogs with congestive
heart failure. *K. B. Margulies, M. A. Perrella,
L. J. McKinley, and J. C. Burnett, Jr.* 1636
- Sjögren-Larsson syndrome. Deficient activity of the fatty-
aldehyde dehydrogenase component of fatty
alcohol:NAD $^+$ oxidoreductase in cultured fibroblasts.
W. B. Rizzo and D. A. Craft 1643
- Evidence for direct estrogen regulation of the human
gonadotropin-releasing hormone gene. *S. Radovick,
C. M. Ticknor, Y. Nakayama, A. C. Notides, A. Rahman,
B. D. Weintraub, G. B. Cutler, Jr., and F. E. Wondisford* 1649

- Constitutive expression of a 92-kD gelatinase (type V collagenase) by rheumatoid synovial fibroblasts and its induction in normal human fibroblasts by inflammatory cytokines. *E. N. Unemori, M. S. Hibbs, and E. P. Amento* 1656
- Flow activates an endothelial potassium channel to release an endogenous nitrovasodilator. *J. P. Cooke, E. Rossitch, Jr., N. A. Andon, J. Loscalzo, and V. J. Dzau* 1663
- Evidence for persistent hepatitis C virus (HCV) infection in hemophiliacs. *J.-P. Allain, S. H. Dailey, Y. Laurian, D. S. Vallari, A. Rafowicz, S. M. Desai, and S. G. Devare* 1672
- Acceleration of the thrombin inactivation of single chain urokinase-type plasminogen activator (pro-urokinase) by thrombomodulin. *G. A. W. de Munk, E. Groeneveld, and D. C. Rijken* 1680
- Factor X_{Santo Domingo}. Evidence that the severe clinical phenotype arises from a mutation blocking secretion. *H. H. Watzke, A. Wallmark, N. Hamaguchi, P. Giardina, D. W. Stafford, and K. A. High* 1685
- Inhibition of platelet function by an aspirin-insensitive endothelial cell ADPase. Thromboregulation by endothelial cells. *A. J. Marcus, L. B. Safier, K. A. Hajjar, H. L. Ullman, N. Islam, M. J. Broekman, and A. M. Eiroa* 1690
- In vitro growth rate of placental fibroblasts is developmentally regulated. *M. E. Fant* 1697
- Differential regulation of Na/H antiporter by acid in renal epithelial cells and fibroblasts. *O. W. Moe, R. T. Miller, S. Horie, A. Cano, P. A. Preisig, and R. J. Alpern* 1703
- Nerve growth factor in the urinary bladder of the adult regulates neuronal form and function. *W. D. Steers, S. Kolbeck, D. Creedon, and J. B. Tuttle* 1709
- Exclusion of linkage between the collagenase gene and generalized recessive dystrophic epidermolysis bullosa phenotype. *A. Hovnanian, P. Duquesnoy, S. Amselem, C. Blanchet-Bardon, M. Lathrop, L. Dubertret, and M. Goossens* 1716
- Molecular and metabolic basis for the metabolic disorder normotriglyceridemic abetalipoproteinemia. *D. A. Hardman, C. R. Pullinger, R. L. Hamilton, J. P. Kane, and M. J. Malloy* 1722
- Erythropoietic protoporphyria in the house mouse. A recessive inherited ferrochelatase deficiency with anemia, photosensitivity, and liver disease. *S. Tutois, X. Montagutelli, V. Da Silva, H. Jouault, P. Rouyer-Fessard, K. Leroy-Viard, J.-L. Guénet, Y. Nordmann, Y. Beuzard, and J.-C. Deybach* 1730
- Accumulation of fetal fibronectin mRNAs during the development of rat cardiac hypertrophy induced by pressure overload. *J. L. Samuel, A. Barrieux, S. Dufour, I. Dubus, F. Contard, V. Koteliensky, F. Farhadian, F. Marotte, J.-P. Thiéry, and L. Rappaport* 1737
- Dysregulation of in vitro cytokine production by monocytes during sepsis. *C. Munoz, J. Carlet, C. Fitting, B. Misset, J.-P. Blériot, and J.-M. Cavaillon* 1747
- ### Rapid Publications
- Reduction in plasma human immunodeficiency virus ribonucleic acid after dideoxynucleoside therapy as determined by the polymerase chain reaction. *M. Holodniy, D. A. Katzenstein, D. M. Israelski, and T. C. Merigan* 1755
- Active site-blocked factor IXa prevents intravascular thrombus formation in the coronary vasculature without inhibiting extravascular coagulation in a canine thrombosis model. *C. R. Benedict, J. Ryan, B. Wolitzky, R. Ramos, M. Gerlach, P. Tijburg, and D. Stern* 1760
- Human Fc_γRII, in the absence of other Fc_γ receptors, mediates a phagocytic signal. *Z. Indik, C. Kelly, P. Chien, A. I. Levinson, and A. D. Schreiber* 1766
- Lactate activates ATP-sensitive potassium channels in guinea pig ventricular myocytes. *E. C. Keung and Q. Li* 1772
- A lymphocyte homing receptor (L-Selectin) mediates the in vitro attachment of lymphocytes to myelinated tracts of the central nervous system. *K. Huang, J. S. Geoffroy, M. S. Singer, and S. D. Rosen* 1778
- November Author Index 1784

- Evidence for direct estrogen regulation of the human gonadotropin-releasing hormone gene. *S. Radovick, C. M. Ticknor, Y. Nakayama, A. C. Notides, A. Rahman, B. D. Weintraub, G. B. Cutler, Jr., and F. E. Wondisford* 1649
- Dysregulation of in vitro cytokine production by monocytes during sepsis. *C. Munoz, J. Carlet, C. Fitting, B. Misset, J.-P. Blériot, and J.-M. Cavaillon* 1747

■ Cell Growth and Differentiation

- The vascular smooth muscle α -actin gene is reactivated during cardiac hypertrophy provoked by load. *F. M. Black, S. E. Packer, T. G. Parker, L. H. Michael, R. Roberts, R. J. Schwartz, and M. D. Schneider* 1581
- Mithramycin inhibits SP1 binding and selectively inhibits transcriptional activity of the dihydrofolate reductase gene in vitro and in vivo. *S. W. Blume, R. C. Snyder, R. Ray, S. Thomas, C. A. Koller, and D. M. Miller* 1613
- In vitro growth rate of placental fibroblasts is developmentally regulated. *M. E. Fant* 1697
- Nerve growth factor in the urinary bladder of the adult regulates neuronal form and function. *W. D. Steers, S. Kolbeck, D. Creedon, and J. B. Tuttle* 1709
- * Human Fc_γRII, in the absence of other Fc_γ receptors, mediates a phagocytic signal. *Z. Indik, C. Kelly, P. Chien, J. Levinson, and A. D. Schreiber* 1766

■ Cellular, Transport and Organ Physiology

- Feedback inhibition of cyclic adenosine monophosphate-stimulated Na⁺ transport in the rabbit cortical collecting duct via Na⁺-dependent basolateral Ca⁺⁺ entry. *M. D. Breyer* 1502
- Effect of denervation on the expression of two glucose transporter isoforms in rat hindlimb muscle. *N. E. Block, J. R. Menick, K. A. Robinson, and M. G. Buse* 1546
- Evidence from oocyte expression that the erythrocyte water channel is distinct from band 3 and the glucose transporter. *R. Zhang, S. L. Alper, B. Thorens, and A. S. Verkman* 1553
- Accumulation of hyaluronan and tissue edema in experimental myocardial infarction. *A. Waldenström, H. J. Martinussen, B. Gerdin, and R. Hällgren* 1622
- Flow activates an endothelial potassium channel to release an endogenous nitrovasodilator. *J. P. Cooke, E. Rossitch, Jr., N. A. Andon, J. Loscalzo, and V. J. Dzau* 1663

- Differential regulation of Na/H antiporter by acid in renal epithelial cells and fibroblasts. *O. W. Moe, R. T. Miller, S. Horie, A. Cano, P. A. Preisig, and R. J. Alpern* 1703
- Accumulation of fetal fibronectin mRNAs during the development of rat cardiac hypertrophy induced by pressure overload. *J. L. Samuel, A. Barrieux, S. Dufour, I. Dubus, F. Contard, V. Koteliensky, F. Farhadian, F. Marotte, J.-P. Thiéry, and L. Rappaport* 1737
- * Lactate activates ATP-sensitive potassium channels in guinea pig ventricular myocytes. *E. C. Keung and Q. Li* 1772

■ Atherosclerosis / Thrombosis / Metabolism

- Influence of apolipoprotein E polymorphism on apolipoprotein B-100 metabolism in normolipemic subjects. *T. Demant, D. Bedford, C. J. Packard, and J. Shepherd* 1490
- Deficiency in phosphorylase phosphatase activity despite elevated protein phosphatase type-1 catalytic subunit in skeletal muscle from insulin-resistant subjects. *B. L. Nyomba, D. L. Brautigan, K. K. Schlender, W. Wang, C. Bogardus, and D. M. Mott* 1540
- von Willebrand factor binding to platelet GpIb initiates signals for platelet activation. *M. H. Kroll, T. S. Harris, J. L. Moake, R. I. Handin, and A. I. Schafer* 1568
- Systemic lysis protects against the effects of platelet activation during coronary thrombolysis. *D. J. Fitzgerald, M. Hanson, and G. A. FitzGerald* 1589
- Acceleration of the thrombin inactivation of single chain urokinase-type plasminogen activator (pro-urokinase) by thrombomodulin. *G. A. W. de Munk, E. Groeneveld, and D. C. Rijken* 1680
- Inhibition of platelet function by an aspirin-insensitive endothelial cell ADPase. Thromboregulation by endothelial cells. *A. J. Marcus, L. B. Safier, K. A. Hajjar, H. L. Ullman, N. Islam, M. J. Broekman, and A. M. Eiroa* 1690
- * Active site-blocked factor IXa prevents intravascular thrombus formation in the coronary vasculature without inhibiting extravascular coagulation in a canine thrombosis model. *C. R. Benedict, J. Ryan, B. Wolitzky, R. Ramos, M. Gerlach, P. Tijburg, and D. Stern* 1760
- November Author Index 1784

* Perspectives article
† Rapid Publication

Cover picture: Shown is a secondary structure model of the Na⁺/glucose cotransporter of human intestinal microvilli, depicting a known N-linked complex of oligosaccharide and 12 postulated membrane spans. In two children of a Syrian family afflicted with glucose/galactose malabsorption a single missense mutation, aspartate (D) to asparagine (N) was found which destroyed transport activity and was shown to be the cause of this autosomal recessive disease. Prepared using CorelDraw2. See related article in this issue by Wright et al., pp. 1435-1440.

The Journal of Clinical Investigation
November 1991, Volume 88, Number 5

Molecular Medicine / Genetic Disorders

- * Molecular genetics of intestinal glucose transport. E. M. Wright, E. Turk, B. Zabel, S. Mundlos, and J. Dyer 1435
- * Genetic causes of aortic aneurysms. Unlearning at least part of what the textbooks say. H. Kuivaniemi, G. Tromp, and D. J. Prockop 1441
- Cytogenetic and molecular genetic studies of follicular and papillary thyroid cancers: M. A. Herrmann, I. D. Hay, D. H. Bartelt, Jr., S. R. Ritland, R. J. Dahl, C. S. Grant, and R. B. Jenkins 1596
- Sjögren-Larsson syndrome. Deficient activity of the fatty aldehyde dehydrogenase component of fatty alcohol:NAD⁺ oxidoreductase in cultured fibroblasts. W. B. Rizzo and D. A. Craft 1643
- Factor X_{Santo Domingo}. Evidence that the severe clinical phenotype arises from a mutation blocking secretion. H. H. Watzke, A. Wallmark, N. Hamaguchi, P. Giardina, D. W. Stafford, and K. A. High 1685
- Exclusion of linkage between the collagenase gene and generalized recessive dystrophic epidermolysis bullosa phenotype. A. Hovnanian, P. Duquesnoy, S. Amselem, C. Blanchet-Bardon, M. Lathrop, L. Dubertret, and M. Goossens 1716
- Molecular and metabolic basis for the metabolic disorder normotriglyceridemic abetalipoproteinemia. D. A. Hardman, C. R. Pullinger, R. L. Hamilton, J. P. Kane, and M. J. Malloy 1722
- Erythropoietic protoporphyria in the house mouse. A recessive inherited ferrochelatase deficiency with anemia, photosensitivity, and liver disease. S. Tutois, X. Montagutelli, V. Da Silva, H. Jouault, P. Rouyer-Fessard, K. Leroy-Viard, J.-L. Guénet, Y. Nordmann, Y. Beuzard, and J.-C. Deybach 1730

Infection / Inflammation / Immunity

- Increased expression of the interleukin 1 receptor on blood neutrophils of humans with the sepsis syndrome. M. B. Fasano, S. Cousart, S. Neal, and C. E. McCall 1452
- * Molecular cloning and characterization of recombinant parasite antigens for immunodiagnosis of onchocerciasis. R. Chandrashekar, K. Masood, R. M. Alvarez, A. F. Ogunrinade, R. Lujan, F. O. Richards, Jr., and G. J. Weil 1460
- Antimalarial effects of peptide inhibitors of a *Plasmodium falciparum* cysteine proteinase. P. J. Rosenthal, W. S. Wollish, J. T. Palmer, and D. Rasnick 1467
- Activation of T lymphocytes in dengue virus infections. High levels of soluble interleukin 2 receptor, soluble CD4, soluble CD8, interleukin 2, and interferon- γ in sera of children with dengue. I. Kurane, B. L. Innis, S. Nimmannitya, A. Nisalak, A. Meager, J. Janus, and F. A. Ennis 1473

- Hydrogen peroxide-mediated toxicity for *Leishmania donovani* chagasi promastigotes. Role of hydroxyl radical and protection by heat shock. J. H. Zarley, B. E. Britigan, and M. E. Wilson 1511
- Mesangial cell autoantigens in immunoglobulin A nephropathy and Henoch-Schönlein purpura. D. J. O'Donoghue, A. Darvill, and F. W. Ballardie 1522
- Escherichia coli* hemolysin is a potent inducer of phosphoinositide hydrolysis and related metabolic responses in human neutrophils. F. Grimminger, U. Sibelius, S. Bhakdi, N. Suttorp, and W. Seeger 1531
- Neutrophil migration across a cultured intestinal epithelium. Dependence on a CD11b/CD18-mediated event and enhanced efficiency in physiological direction. C. A. Parkos, C. Delp, M. A. Arnaout, and J. L. Madara 1605
- Constitutive expression of a 92-kD gelatinase (type V collagenase) by rheumatoid synovial fibroblasts and its induction in normal human fibroblasts by inflammatory cytokines. E. N. Unemori, M. S. Hibbs, and E. P. Amento 1656
- Evidence for persistent hepatitis C virus (HCV) infection in hemophiliacs. J.-P. Allain, S. H. Dailey, Y. Laurian, D. S. Vallari, A. Rafowicz, S. M. Desai, and S. G. Devare 1672
- * Reduction in plasma human immunodeficiency virus ribonucleic acid after dideoxynucleoside therapy as determined by the polymerase chain reaction. M. Holodniy, D. A. Katzenstein, D. M. Israelski, and T. C. Merigan 1755
- * A lymphocyte homing receptor (L-Selectin) mediates the in vitro attachment of lymphocytes to myelinated tracts of the central nervous system. K. Huang, J. S. Geoffroy, M. S. Singer, and S. D. Rosen 1778

Hormones / Cytokines / Signaling

- * Interleukin 1 receptor antagonist. A new member of the interleukin 1 family. W. P. Arend 1445
- Failure of atrial natriuretic factor to increase with saline load in patients with dilated cardiomyopathy and mild heart failure. M. Volpe, C. Tritto, N. De Luca, A. F. Mele, G. Lembo, S. Rubattu, M. Romano, P. De Campora, I. Enea, B. Ricciardelli, B. Trimarco, and M. Condorelli 1481
- L-Arginine abrogates salt-sensitive hypertension in Dahl/Rapp rats. P. Y. Chen and P. W. Sanders 1559
- Regulation of transforming growth factor- β 1 gene expression by glucocorticoids in normal human T lymphocytes. O. AyanlarBatuman, A. P. Ferrero, A. Diaz, and S. A. Jimenez 1571
- Differences in insulin action as a function of original anatomical site of newly differentiated adipocytes obtained in primary culture. C. Szalryd, S. Azhar, and G. M. Reaven 1629
- Angiotensin inhibition potentiates the renal responses to neutral endopeptidase inhibition in dogs with congestive heart failure. K. B. Margulies, M. A. Perrella, L. J. McKinley, and J. C. Burnett, Jr. 1636

(continued on the reverse side of this cover)

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Inhibition of Platelet Function by an Aspirin-insensitive Endothelial Cell ADPase Thromboregulation by Endothelial Cells

Aaron J. Marcus, Lenore B. Safier, Katherine A. Hajjar, Harris L. Ullman, Naziba Islam, M. Johan Broekman, and Ana M. Eiroa
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Abstract

We previously reported that platelets become unresponsive to agonists when stimulated in combined suspension with aspirin-treated human umbilical vein endothelial cells. Inhibition occurred concomitant with metabolism of platelet-derived endoperoxides to prostacyclin by endothelial cells. We now demonstrate that if aspirin-treated platelets which fully respond to appropriate doses of agonists are exposed to aspirin-treated endothelial cells, they remain unresponsive despite absence of prostacyclin. Platelet inhibition is due in large part to ecto-ADPase activity on the endothelial cells. This was established by incubating aspirin-treated endothelial cells with ^{14}C -ADP. Radio-thin layer chromatography and aggregometry demonstrated that ^{14}C -ADP and induction of platelet activation decreased rapidly and concurrently. AMP accumulated transiently, was further metabolized to adenosine, and deaminated to inosine. The apparent K_m of the endothelial cell ADPase was $33\text{--}42\text{ }\mu\text{M}$ and the V_{\max} $17\text{--}43\text{ nmol/min per }10^6\text{ cells}$, values in the range of antithrombotic potential.

Thus, at least three complementary systems in human endothelial cells control platelet responsiveness: a cell-associated, aspirin-insensitive ADPase which functions in parallel with fluid phase autacoids such as the aspirin-inhibitable eicosanoids, and the aspirin-insensitive endothelium-derived relaxing factor. (*J. Clin. Invest.* 1991;88:1690–1696.) Key words: nucleotidases • cell-cell interactions • platelet aggregation • platelet serotonin release • thrombosis

Introduction

Realization of the importance of vascular cell-cell interactions and transcellular metabolism has increased in recent years (1). This is particularly pertinent to the case of endothelial cells and platelets. Currently we hypothesize that endothelial cells control platelet reactivity via at least three mechanisms: a cell-associated ADPase system and two fluid phase reactants; eicosanoids such as prostacyclin (PGI_2)¹; and the endothelium-derived relaxing factor (EDRF). In this report we extend previous

studies on platelet inhibition by PGI_2 formed by aspirin-treated endothelial cells from platelet endoperoxides (2). Under experimental conditions in which EDRF was not measurable, we found that platelet reactivity was inhibited by endothelial cells even though both cell types were aspirin treated and PGI_2 was absent. Biochemical and functional data will be presented indicating that these aspirin-treated endothelial cells inhibit platelet function largely via a mechanism involving metabolism of ADP and consequent loss of its proaggregatory activity.

Methods

Preparation of platelet-rich plasma and platelet suspensions. Blood was collected from donors ~ 12 h after they had ingested 650 mg acetylsalicylic acid, aspirin (ASA). Platelet-rich plasma (PRP) was prepared using acid citrate-dextrose (citric acid, 38 mM; sodium citrate, 75 mM; glucose, 135 mM) as anticoagulant (3), with an initial whole blood centrifugation at 200 g, 15 min (25°C) and a second centrifugation of the PRP (90 g, 10 min) to eliminate most of the residual erythrocytes and leukocytes. PRP was maintained at room temperature under 5% CO_2 -air.

Platelet suspensions, when used, were prepared from PRP as described previously (3). Final resuspension was in cold 0.15 M NaCl to a count of 1×10^8 platelets/ $20\text{ }\mu\text{l}$. The suspension was kept in a closed container at 4°C .

Preparation of endothelial cell suspensions. Cultured human endothelial cells (P2-P8) derived from umbilical cords (4) were treated with 1 mM ASA ($10\text{ }\mu\text{l}$ of 1 M ASA in ethanol/ 10 ml) for 30 min at 37°C (2). Cells were washed in Hepes-buffered saline and detached with collagenase-EDTA-BSA solution (4). An equal volume of human serum-containing medium was added, the cells centrifuged at 500 g for 10 min (22°C), and finally resuspended in ASA-free buffer ($0.25\text{ ml/T-75 flask}$). Resuspension buffer was either Tris-Saline-Glucose (TSG) (Tris, 15 mM; NaCl, 134 mM; glucose, 5 mM, pH 7.4) or Hepes, 5 mM; NaCl, 140 mM; KCl, 5 mM; CaCl_2 , 1.29 mM; MgCl_2 , 1.20 mM, pH 7.45. Indomethacin was then added to a concentration of $10\text{ }\mu\text{M}$. Suspensions were generally maintained at room temperature or at 4°C as specified. Endothelial cell (EC) counts averaged 4,466 cells/ μl .

Aggregation studies with combined suspensions of ASA-treated platelets and ASA-treated EC. These experiments were carried out similarly to those previously reported in which ASA-treated EC, but untreated platelets were studied (2). ASA-PRP containing 1×10^8 platelets or ASA-treated washed platelets (1×10^8) in buffer were preincubated (3 min, 37°C) in siliconized cuvettes containing stirring bars in an aggregometer. When used, SOD or other substances of interest such as hemoglobin, FeSO_4 , or nitroprusside were included with the platelets or during preincubation. ASA-treated EC were then added, followed in 1 min by the agonist. In the case of TSG buffer, which contained no calcium, Ca^{++} (3 mM) was included 15 s before thrombin or collagen. Total volumes were adjusted to $500\text{ }\mu\text{l}$ with buffer. The aggregation response was recorded over a 5-min period in a Lumiaggregometer (Chrono-Log Corp., Havertown, PA). Control "platelet-poor" cuvettes contained equal numbers of EC to those in "platelet-rich" cuvettes in order to correct for light absorption by the nonaggregating EC.

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1. Abbreviations used in this paper: ASA, acetylsalicylic acid, aspirin; EC, endothelial cell(s); EDRF, endothelium-derived relaxing factor; 5-HT, serotonin, 5-hydroxytryptamine; NO, nitric oxide; PGI_2 , prostacyclin; PRP, platelet-rich plasma.

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Studies of possible inhibitory properties of ASA-EC supernatants. EC were tested for their inhibitory activity as above, using ADP, thrombin, or collagen as platelet agonist. Separate aliquots of EC were equilibrated at 37°C in polypropylene microfuge tubes with stirring (in the presence or absence of SOD, 60 U/ml). They were then incubated (15 s) with specific EC stimuli such as bradykinin (100 nM), acetylcholine (2 μ M), histamine (10 μ M), or thrombin (1.25 U/ml), followed by rapid centrifugation (10 s, 15,600 g; Eppendorf Inc., Fremont, CA). Supernatants were transferred to aggregometer cuvettes containing 1×10^8 ASA-treated platelets (either washed or in PRP). Platelet agonists (ADP, collagen, or thrombin; see below) were added 15–20 s later. Total volumes in the cuvettes were adjusted to 500 μ l with buffer. Controls were carried out to evaluate effects of carry-over of EC agonists on platelet aggregation. In the case of thrombin, the quantity carried over served as platelet agonist in the cuvette.

Time course of EC ADPase activity. ASA-EC in a total volume of 400 μ l, plus 15 μ M (14 C) ADP (41.7 mCi/mmol; New England Nuclear, Boston, MA), or buffer plus (14 C) ADP for controls, were incubated as in the other centrifugation studies for varying periods of time (15 s–30 min). Stirring bars were rapidly removed and the tubes centrifuged as above. A 100- μ l aliquot of supernatant was transferred to the cuvette containing platelets and the aggregation response recorded. In control experiments containing no EC, the final ADP concentration in the cuvettes was 3 μ M.

Immediately after removal of the initial 100 μ l of supernatant, an additional 200 μ l was placed in a polypropylene microfuge tube containing 10 μ l of "stop solution" (5). The stop solution stock consisted of 160 mM disodium EDTA, neutralized to pH 7, plus 17 mM ADP in ice-cold physiological saline. The stop solution was added to prevent any possible further degradation of ADP. Tubes were then vortexed and kept on ice until the experiment was concluded. Scintillation counting was performed on 5 μ l from each sample. Tubes were stored at –70°C for thin-layer chromatography of ADP and its metabolites.

Incubations could also be carried out with endothelial cell monolayers in multiwell plates (well capacity 1.5 ml, growth area 2 cm²; 3847; Falcon Labware, Becton Dickinson & Co., Lincoln Park, NJ), without stirring, using the same ADP concentration and total volume. Plates were secured by weights in a 37°C water bath. At indicated time intervals, supernatants were aspirated and centrifuged as above.

The well system was also used to determine the ADPase activity of fresh endothelial cells. Cells derived from two cords were pooled and divided between nine wells. Within 1–2 h of incubation, the endothelial cells were adherent. This permitted assay of EC ADPase activity free from contaminating erythrocytes, which were discarded in the supernatant.

Aggregation studies using supernatants of either thrombin-stimulated platelets or thrombin-stimulated platelet-endothelial cell mixtures as platelet agonist. Washed platelets (1×10^8) from a donor who had ingested aspirin, or platelets plus 2×10^6 ASA-treated endothelial cells were preincubated for 3 min and then stimulated with thrombin (1 U/ml), in the presence of 3 mM Ca⁺⁺ and 20 μ M hemoglobin. Total incubation volume was 400 μ l. The microfuge tubes were incubated for 5 min after stimulation, stirring bars removed and centrifugation carried out for 10 s (Eppendorf). A control tube containing thrombin but no cells was also incubated for 5 min.

100 μ l of supernatant was rapidly transferred to an aggregometer cuvette containing 227 μ l of PRP from the same donor (1.3×10^8 platelets) and buffer (total volume of the assay cuvette was 500 μ l). The aggregation response was recorded for 4 min.

TLC studies of nucleotides, nucleosides, and bases. TLC was carried out on fluorescent silica gel 60 F₂₅₄-coated plastic sheets (20 \times 20 cm; EM Separations, Gibbstown, NJ). 15 μ l of radioactive sample was applied and dried under a stream of air. At each point of application, 2 μ l of a mixture of standards was added and dried. The standard mixture consisted of 2 mg each of the following compounds: ATP, ADP, AMP, inosine, hypoxanthine, adenosine, and adenine in distilled water (total vol = 1 ml) (6). The solvent system for nucleotides, nucleosides, and bases consisted of isobutyl alcohol/1-pentanol/ethylene gly-

col monoethyl ether/NH₄OH/H₂O (90:60:180:90:120) (solvent 1) (6). For separation of hypoxanthine and adenosine, the solvent system consisted of 1-butanol/ethyl acetate/methanol/NH₄OH (7:4:3:4) (solvent 2) (7).

Solvent systems were prepared at least 48 h before use and added to the tank 1 h before insertion of plates (8). Development of plates was carried out for 5 h, 10 min in solvent 1, or 4 h in solvent 2. Plates were dried under a stream of warm air, the separated compounds visualized under ultraviolet light (254 nm), and scanned for radioactivity with an RTLC multi-scanner (Radiomatic Instruments & Chemical Co., Inc., Tampa, FL).

Substrate concentration curve of EC ADPase. Aspirin-treated EC (72,233/400 μ l total vol) in TSG buffer were incubated with stirring for 5 min (37°C) with 1.2–80.1 μ M ADP containing 2.4 μ Ci of (3 H) ADP (trisodium salt, 27.3 Ci/mmol; New England Nuclear), in the presence of 10 μ M dipyridamole. The latter was used to prevent reuptake of adenosine and consequent resynthesis of ADP (7, 9). Ca⁺⁺ (1.22 mM) was added 30 s before ADP. As in the time course experiments described above, tubes were then centrifuged, supernatants treated with stop solution, aliquots counted for total radioactivity, and TLC performed. The dipyridamole stock solution was prepared in glycine-HCl buffer (0.05 M, pH 2.8). During these experiments, EC stock suspensions were stored at 4°C.

(14 C) Serotonin, 5-hydroxytryptamine (5-HT) release. For platelet labeling, 0.2 nmol (14 C) 5-HT creatinine sulfate (54 mCi/mmol; Amersham Corp., Arlington Heights, IL) was added directly to the anticoagulant for each ml of anticoagulated blood. (14 C) 5-HT uptake was determined 1 h after blood collection by comparison of radioactivity in 50- μ l aliquots of PRP and platelet-poor plasma. Assays of the effects of EC on platelet (14 C) 5-HT release and aggregation were carried out in cuvettes in the usual manner, using 1×10^8 labeled platelets in suspension or PRP. To prevent reuptake of released 5-HT, imipramine (2.5 μ M) was added 90 s before the agonist. Controls containing labeled platelets without agonist were carried out to measure any release of (14 C) 5-HT attributable to stirring alone. Reactions were stopped 4 min after stimulation by placing the cuvettes on ice. Cuvette contents were transferred to microfuge tubes and centrifuged for 3 min (10,000 g, 4°C). 50- μ l aliquots of each supernatant were counted in 4 ml Aquasol-2 and compared to total platelet counts.

Additional methods. Treatment of platelets with methylene blue, an inhibitor of soluble guanylate cyclase, was essentially according to Alheid et al. (10). Methylene blue was added to a washed platelet suspension at a concentration of 10 μ M. The suspension was then left at room temperature for 30 min, centrifuged at 1,450 g (15 min, 4°C) and the pellet resuspended in cold 0.15 M NaCl.

Oxyhemoglobin was prepared from bovine hemoglobin (type 1; Sigma Chemical Co., St. Louis, MO) by the method of Martin et al. (11). Human hemoglobin yielded identical results. Briefly, a 1-mM solution of the commercial mixture of oxyhemoglobin and methemoglobin was reduced with a 10-fold molar excess of sodium dithionite, which was then removed by dialysis. Purity was checked spectrophotometrically and aliquots frozen at –70°C.

Adenosine-5'-O-(2-thiodiphosphate) trilithium salt (ADP- β -S) was obtained from Boehringer Mannheim, Indianapolis, IN.

Prostacyclin production was measured by RIA for 6-keto-PGF_{1 α} (DuPont-New England Nuclear).

Results

Endothelial cells inhibit platelet reactivity in totally aspirin-treated systems. When ASA-treated washed platelets were stimulated by agonists in the presence of ASA-treated endothelial cells, platelet aggregation was inhibited (Fig. 1). This occurred under conditions where control ASA-platelets alone were fully aggregated by the same quantity of stimulus (Fig. 1). RIA measurements verified that no PGI₂ had formed in these experi-

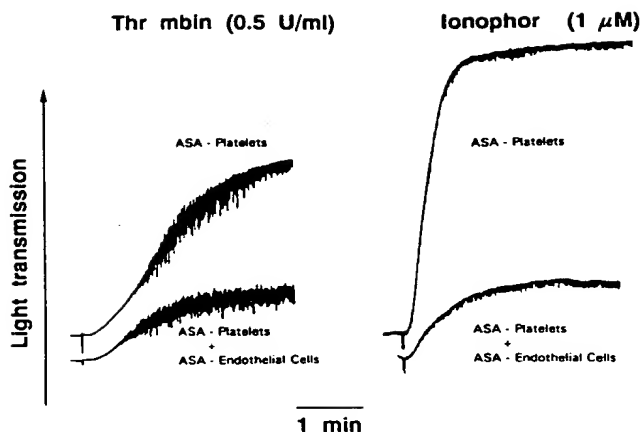


Figure 1. Inhibition of platelet aggregation by endothelial cell suspensions. The upper curves (controls) represent the response of washed, aspirin-treated platelets to thrombin and ionophore, respectively. The lower curves depict inhibited platelet responsiveness when aspirin-treated endothelial cells were present. Aspirin treatment of both cell types prevented transcellular metabolism of platelet endoperoxide to prostacyclin by the endothelial cells (2).

ments. As shown in Fig. 2 A, PRP from a donor who had ingested aspirin was fully aggregated by $1 \mu\text{M}$ ADP. In contrast, when this PRP was stimulated in the presence of 1×10^6 ASA-EC, the inhibited aggregation curve was characterized by a brief ascending limb followed by reversal. The pattern of reversibility was reminiscent of previous experiments in this laboratory wherein ADP released from platelets by PGH_2 was intentionally removed by enzymatic means (apyrase) (Fig. 2 B).

Concurrent metabolism of ADP by endothelial cells and loss of its potential as a platelet agonist. The hypothesis that an ADPase activity was present on these human endothelial cells and could possibly account for their platelet-inhibitory properties was tested biochemically and functionally. (^{14}C) ADP ($15 \mu\text{M}$) was incubated with ASA-EC for increasing periods of time. The supernatants were then examined for their content of residual (^{14}C) ADP and its metabolites, as well as for the platelet aggregating potential of the unmetabolized (^{14}C) ADP. Re-

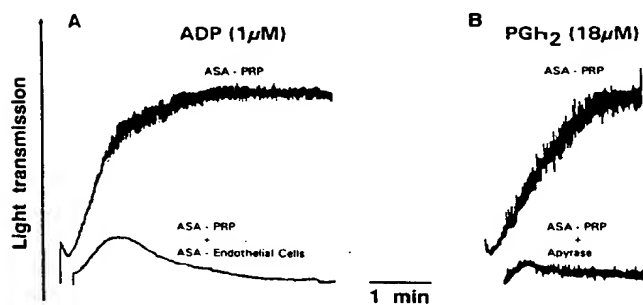


Figure 2. Comparison of the inhibition of ADP-induced aggregation in PRP by endothelial cells (A), with that due to enzymatic removal by apyrase of platelet ADP released by prostaglandin endoperoxide (PGH_2) stimulation (B). Similarities in the shapes of the curves of inhibited aggregation (lower curves) suggest enzymatic removal of ADP in A as well.

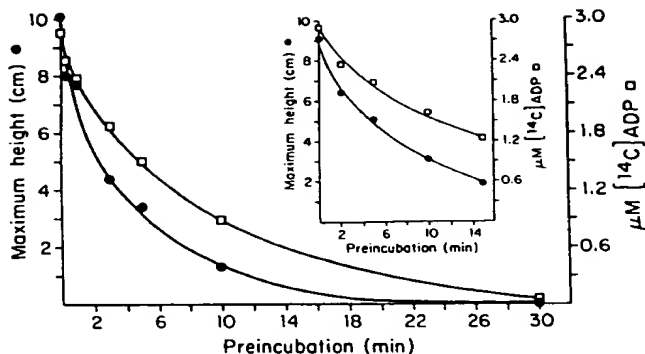


Figure 3. Time course of metabolism of (^{14}C) ADP by aspirin-treated endothelial cell suspensions. The decrease in (^{14}C) ADP concentration as measured by thin-layer radiochromatography (\square), was accompanied by loss of platelet proaggregatory activity (\bullet) of supernatants derived from incubation of endothelial cells with (^{14}C) ADP. This also occurred when endothelial cell monolayers were used (inset). Results shown are from representative experiments in which 72,215 suspended EC or 84,625 EC in monolayers were assayed.

sults are depicted in Figs. 3 and 4. When compared to (^{14}C) ADP controls which had been incubated with buffer alone, the presence of EC resulted in a progressive decrease in (^{14}C) ADP concentration. This was paralleled by loss of supernatant proaggregatory activity as measured by the decrease in maximum height of the platelet aggregation curves. Comparable results were obtained whether endothelial cell suspensions (Fig. 3) or monolayers (Fig. 3, inset) were employed. ADPase activity was also present on freshly adherent, but uncultured endo-

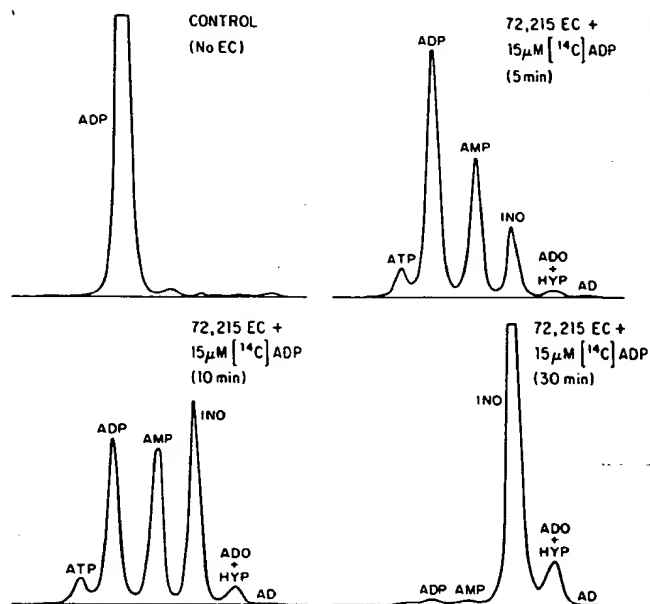


Figure 4. Time course of formation of metabolites of (^{14}C) ADP by aspirin-treated endothelial cell suspensions. Cell-free supernatants derived from incubations of endothelial cells with (^{14}C) ADP were analyzed for (^{14}C) ADP and its metabolites by TLC.

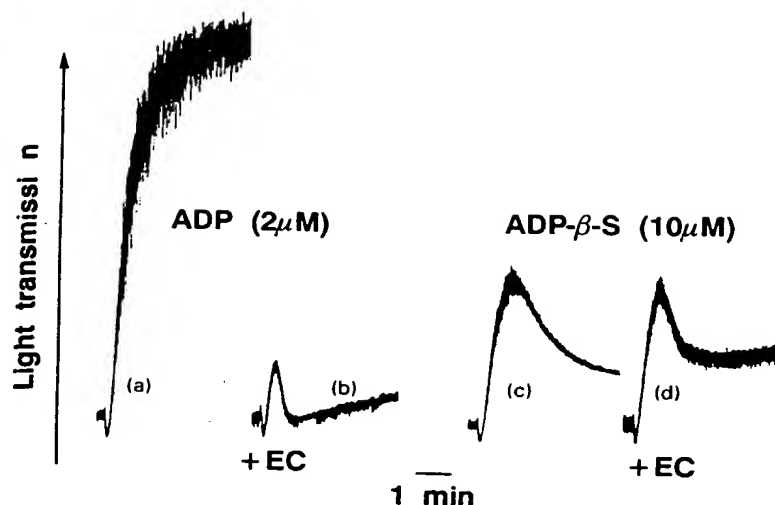


Figure 5. Studies with ADP- β -S, a nonmetabolizable ADP analogue that activates platelets. (a) ADP elicited a full aggregation response in aspirin-treated platelets. (b) Inhibition of the ADP response by aspirin-treated endothelial cells. (c) Aggregation response of aspirin-treated platelets to ADP- β -S. (d) Aggregation induced by ADP- β -S was not appreciably influenced by the presence of endothelial cells, which were unable to metabolize the ADP- β -S.

thelial cells. There was only a slight decrease in ADPase activity concomitant with cell passage. Fig. 4 depicts radio-TLC scans of the metabolites of (14 C) ADP after 5, 10, and 30 min incubation with ASA-EC suspensions. At the 5-min time point, ADP had decreased to 51% of total nucleotides, nucleosides, and bases. In addition, AMP (28%) and inosine (13%) were identified, together with trace quantities of adenosine, hypoxanthine, and adenine. By 10 min, concentrations of ADP, AMP, and inosine averaged 30%, 29%, and 29%, respectively. Inosine (85%) was the major (14 C) ADP metabolite at the 30-min interval, and ADP itself was virtually absent. Chromatography with solvent system 2 indicated that hypoxanthine (8%) had been synthesized. As can be seen in Fig. 3, the absence of ADP correlated with total loss of aggregatory activity of the ASA-EC supernatant.

The presence or absence of endothelial cells did not affect the recovery of total radioactivity in supernatants. This indicated that nonspecific adsorption of ADP to the endothelial cells was not the cause of the decrease in supernatant proaggregatory activity.

Endothelial cell inhibition of ADP-induced platelet reactivity requires ADP hydrolysis. Experiments were carried out with ADP- β -S, a structural analogue of ADP that activates platelets, but is not metabolized by ADPases (12). As shown in Fig. 5, ASA-platelets stimulated alone with 2 μ M ADP demonstrated a full aggregation response (a). In the presence of 1×10^6 ASA-EC, this response was markedly attenuated and reversed (b). ADP- β -S (10 μ M) induced a reversible aggregation response of lesser amplitude than ADP (2 μ M) with ASA-platelets alone (c). However, in contrast to results with ADP, ASA-EC did not exert an appreciable inhibitory effect on ADP- β -S-stimulated ASA-platelets (d). These results provided further evidence for ADP hydrolysis by EC as a major mechanism underlying their inhibitory effect on stimulated platelets.

Kinetic parameters of human endothelial cell ADPase. We measured the rate of hydrolysis of (3 H) ADP by ASA-EC as a function of substrate concentration. Apparent K_m and V_{max} values were determined from Lineweaver-Burk plots of the data (Fig. 6). In two experiments, the K_m ranged from 33 to 42 μ M and the V_{max} from 17 to 43 nmol/min per 10^6 cells. These

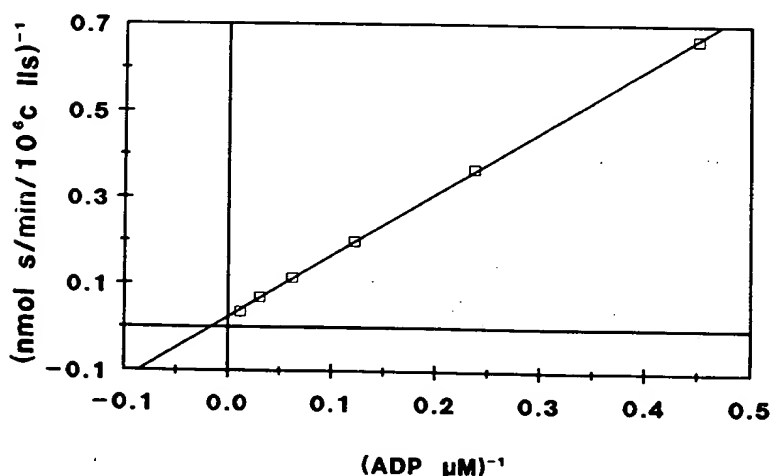


Figure 6. Lineweaver-Burk plot relating the rate of (14 C) ADP hydrolysis by aspirin-treated endothelial cells to ADP concentration. The K_m calculated from these data was 33 μ M and the V_{max} was 42.5 nmol/min per 10^6 cells. Results depicted are representative of two separate experiments.

kinetic parameters are compared in Table I with values reported in the literature for porcine aortic endothelial cells (13, 14).

Endothelial cell ADPase inhibits platelet reactivity independent of EDRF. ASA-endothelial cells (which did not produce PGI₂), inhibited platelet aggregation by an aspirin-insensitive mechanism. This inhibition could have been due in part to a fluid phase reactant such as EDRF in combination with the EC ADPase activity described above. To test this possibility, known modulators of EDRF activity were added to the platelet-EC incubations. Inhibition of platelet aggregation by endothelial cells was not affected by: (a) hemoglobin or ferrous ion, which inactivate EDRF/nitric oxide (NO); (b) methylene blue, which prevents the effects of EDRF/NO by inhibiting soluble guanylate cyclase; or (c) superoxide dismutase, a potentiator of EDRF/NO via removal of superoxide radicals.

Supernatants of EC that had been previously stimulated (15 s) with bradykinin, acetylcholine, or histamine were rapidly transferred to cuvettes containing platelets. There was little (< 10%) if any inhibition of ADP, thrombin, or collagen-stimulated platelet aggregation. This indicated that the platelet inhibitory activity observed in the presence of ASA-endothelial cells was not transferable, and therefore was mainly surface associated. When endothelial cells were prestimulated with thrombin, the quantity present in the transferred supernatant served as agonist in the aggregometry cuvette. Since the same degree of aggregation was obtained with this supernatant (even after a 3-min incubation of thrombin with EC) as with thrombin alone, nonspecific adsorption of agonist to the EC could again be ruled out as the possible etiology of platelet inhibition.

Release of (¹⁴C) 5-HT from stimulated platelets was used as another parameter to gauge the effects of ASA-endothelial cells on platelet reactivity. ASA-endothelial cells inhibited 5-HT release from activated platelets as well as their aggregation (Table II). Hemoglobin did not reverse the inhibitory effect of the ASA-endothelial cells on either 5-HT release or aggregation.

Endothelial cells reverse the capacity of thrombin-elicited platelet releasates to enhance platelet aggregation. To more closely relate the loss of thrombin-induced proaggregatory ac-

Table II. Inhibition of Serotonin Release from Stimulated Aspirin-treated Platelets by Aspirin-treated Endothelial cells

	Serotonin release (%)	
	Collagen 10 µg/ml	Thrombin 0.3 U/ml
Platelets alone	68	66
+ 1 × 10 ⁶ endothelial cells	26	0
+ 1 × 10 ⁶ endothelial cells + hemoglobin (20 µM)	15	4
+ 0.5 × 10 ⁶ endothelial cells	48	—
+ 0.5 × 10 ⁶ endothelial cells + hemoglobin (20 µM)	31	—

Values represent radioactive serotonin released into the supernatant, as percent of total radioactive serotonin in the labeled platelets. Hemoglobin was used as an inhibitor of EDRF/NO activity. If the inhibition of serotonin release were due to EDRF/NO, the inhibition of release would have been reversed in the presence of hemoglobin. These results are representative of four separate experiments.

tivity to EC ADPase action, the following experiments were performed. Supernatants from platelets that had been stimulated with thrombin (1 U/ml) in the absence or presence of endothelial cells were used as agonists for platelet aggregation. Curve (a) in Fig. 7 was generated by the threshold level of thrombin (0.2 U/ml) transferred from the incubation tube to the aggregometry assay cuvette. The enhanced aggregation response in curve (c) was evoked by the combined presence of transferred thrombin-induced platelet releasate in addition to transferred thrombin in the supernatant. Comparable synergy was demonstrated in control experiments in which thrombin (0.2 U/ml) plus concentrations of ADP expected to be released during the initial incubation were added directly to the aggregometer cuvette (data not shown). The presumed concentration of ADP released into the incubation tube, calculated from values reported in the literature (15) was 6.25 µM. The kinetic parameters we derived for the human endothelial cell ADPase under study indicate that this quantity of ADP would have been hydrolyzed by the 2 × 10⁶ EC present during our 5-min incubation. It can be seen in curve (b) Fig. 7 that the presence of EC did indeed reverse the enhanced aggregation.

Discussion

In our earlier studies of platelet responsiveness to agonists when in combined suspension with human endothelial cells, we correlated the observed inhibition of platelet aggregation with prostacyclin formation (2). Under those experimental conditions, the endothelial cells had been pretreated with aspirin, but the platelets were not. Prostacyclin formation was demonstrated to result from metabolism of platelet-derived endoperoxides by endothelial cells in apposition. In the last decade, at least two endothelial cell-associated platelet inhibitory substances in addition to PGI₂ have been identified. These are endothelial cell ecto-ADPases (9, 16) and the endothelium-derived relaxing factor (EDRF/NO) (17–20). Production of EDRF and the action of endothelial cell ADPases are insensitive to aspirin and could have contributed at least in part to results observed in earlier studies (2).

Table I. Kinetic Parameters for Endothelial Cell ADPases from Different Sources

	K_m	V_{max}	V_{max}/K_m
	µM	nmol/min per 10 ⁶ cells	
Human umbilical vein endothelial cells	38	30	0.789
Porcine aortic endothelial cells (13)	155	9.2	0.059
Porcine aortic endothelial cells (14)	247	6.2	0.025

Aspirin-treated endothelial cells were incubated with (³H) ADP. After 5 min the tubes were centrifuged and thin-layer chromatography performed on the supernatants. The rate of hydrolysis of ADP was measured as a function of substrate concentration. Apparent K_m and V_{max} values were determined from Lineweaver-Burk plots as shown in Fig. 6. Values for human umbilical vein endothelial cells represent averages from two separate experiments. These parameters are compared with values reported in the literature.

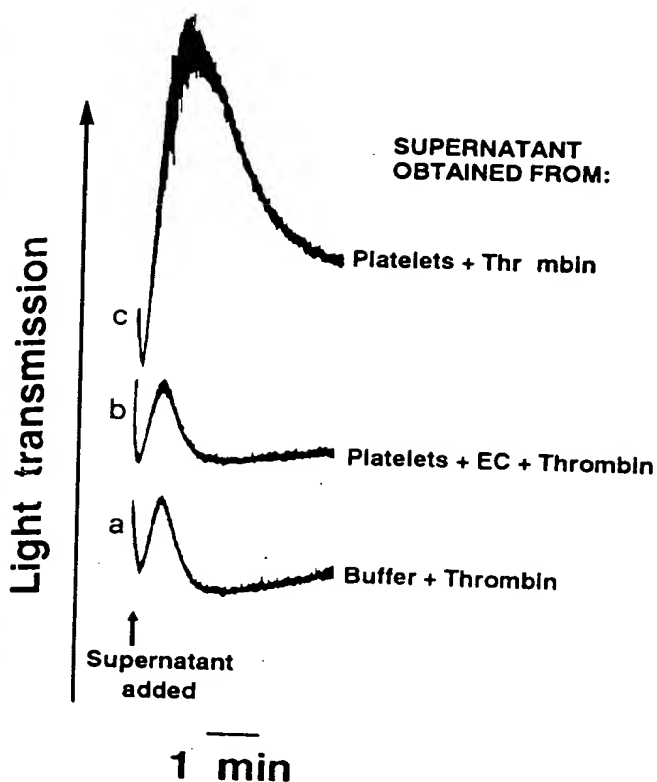


Figure 7. Aggregation response of platelets in PRP to supernatants derived from thrombin-stimulated platelets or thrombin-stimulated platelet-endothelial cell mixtures. (a) Aggregation due to thrombin carried over in the supernatant of a buffer control which contained no cells. In (c) the presence of thrombin-induced platelet releasate in the supernatant produced an enhanced aggregation response. The enhancement of supernatant aggregatory potency as shown in (c) was reversed by the interaction of endothelial cell ADPase with the platelet releasate as shown in (b).

We therefore performed experiments using both aspirin-treated platelets and aspirin-treated endothelial cells, thereby eliminating production of any cyclooxygenase-derived eicosanoids. As shown in Figs. 1 and 2 and Table II, endothelial cells inhibited platelet responsiveness to all agonists tested even when the entire system was aspirin-treated. The endothelial cell-induced inhibition was cell associated since the inhibitory activity was not present in supernatants from endothelial cells even when they had been stimulated with known agonists for EDRF/NO. Substances known to reverse or enhance EDRF/NO activity had no influence on the inhibitory effects of endothelial cells on the platelet responsiveness observed in these experiments. This is shown for hemoglobin in Table II.

When ADP-induced platelet aggregation was reversed in the presence of endothelial cells, the shape of the recorded pattern of reversal suggested that ADP had been enzymatically removed (Fig. 2). In this regard, it was possible to biochemically and functionally correlate metabolism of ADP by ASA-EC with disappearance of its properties as an agonist for platelet aggregation (Figs. 3 and 4). The fact that similar activity was demonstrable with either endothelial cell suspensions or adherent monolayers suggests that the ADPase activity is located on

the luminal surface of the vessel and would interface directly with platelets. Comparable results have been reported by Crutchley and associates with bovine pulmonary arterial endothelial cells (7) and Glasgow et al. with human endothelial cell monolayers (21).

As shown in Table I, values obtained with our human endothelial cell preparations for the apparent K_m and catalytic efficiency (V_{max}/K_m) with ADP as substrate, compared favorably with those reported for porcine aortic endothelial cells (13, 14). Calculations using these figures indicate that the quantity of ADP hydrolyzable by our endothelial cell suspensions would be sufficient to result in inhibition of platelet aggregation. For example, 0.5×10^6 EC would metabolize $2 \mu M$ ADP to $0.8 \mu M$ in 30 s.

In the case of agonists other than ADP, such as thrombin, collagen, and ionophore, hydrolysis of released platelet ADP may also be involved in the loss of platelet responsiveness (Fig. 1, Table II). Calculations based on results depicted in Fig. 7 indicated that enhancement of threshold thrombin aggregation was attributable to thrombin-released platelet ADP. Removal of this ADP by endothelial ecto-ADPase reversed the enhancement, and aggregation reverted to the original threshold level. This occurred in the total absence of endothelial cell PGI_2 and EDRF and emphasizes the role of endothelial cell ADPases in control of platelet recruitment.

A major reason for elucidating biochemical and functional properties of endothelial cell-associated ecto-ADPases is that they can exert a significant effect on platelet reactivity independent of the action of other known endothelium derived inhibitors. For evaluation of the entire known spectrum of EC control of platelet reactivity ("thromboregulation"), cell preparations which simultaneously produce PGI_2 , EDRF/NO, and possess ADPase activity will require additional assessment. It is also possible that as yet undefined endothelial cell thromboregulators could have contributed to results obtained by ourselves and others.

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References

1. Marcus, A. J. 1990. Thrombosis and inflammation as multicellular processes. Pathophysiological significance of transcellular metabolism. *Blood*. 76:1903-1907.
2. Marcus, A. J., B. B. Weksler, E. A. Jaffe, and M. J. Broekman. 1980. Synthesis of prostacyclin from platelet-derived endoperoxides by cultured human endothelial cells. *J. Clin. Invest.* 66:979-986.
3. Marcus, A. J. 1990. Eicosanoid interactions between platelets, endothelial cells and neutrophils. *Methods Enzymol.* 187:585-599.
4. Hajjar, K. A., P. C. Harpel, E. A. Jaffe, and R. L. Nachman. 1986. Binding of plasminogen to cultured human endothelial cells. *J. Biol. Chem.* 261:11656-11662.

5. L  thje, J., A. Schomburg, and A. Ogilvie. 1988. Demonstration of a novel ecto-enzyme on human erythrocytes, capable of degrading ADP and of inhibiting ADP-induced platelet aggregation. *Eur. J. Biochem.* 175:285-289.
6. Cooper, D. R., G. P. Lewis, G. E. Lieberman, H. Webb, and J. Westwick. 1979. ADP metabolism in vascular tissue, a possible thrombo-regulatory mechanism. *Thromb. Res.* 14:901-914.
7. Crutchley, D. J., U. S. Ryan, and J. W. Ryan. 1980. Effects of aspirin and dipyridamole on the degradation of adenosine diphosphate by cultured cells derived from bovine pulmonary artery. *J. Clin. Invest.* 66:29-35.
8. Norman, G. A., M. J. Follett, and D. A. Hector. 1974. Quantitative thin-layer chromatography of ATP and the products of its degradation in meat tissue. *J. Chromatogr.* 90:105-111.
9. Pearson, J. D., J. S. Carleton, and J. L. Gordon. 1980. Metabolism of adenine nucleotides by ectoenzymes of vascular endothelial and smooth-muscle cells in culture. *Biochem. J.* 190:421-429.
10. Alheid, U., J. C. Fr  lich, and U. F  rstermann. 1987. Endothelium-derived relaxing factor from cultured human endothelial cells inhibits aggregation of human platelets. *Thromb. Res.* 47:561-571.
11. Martin, W., G. M. Villani, D. Jothianandan, and R. F. Furchgott. 1985. Selective blockade of endothelium-dependent and glyceryltrinitrate-induced relaxation by hemoglobin and methylene blue in rabbit aorta. *J. Pharmacol. Exp. Ther.* 232:708-716.
12. Goody, R. S., F. Eckstein, and R. H. Schirmer. 1972. The enzymatic synthesis of thiophosphate analogs of nucleotides. *Biochim. Biophys. Acta.* 276:155-161.
13. Cusack, N. J., J. D. Pearson, and J. L. Gordon. 1983. Stereoselectivity of ectonucleotidases on vascular endothelial cells. *Biochem. J.* 214:975-981.
14. Gordon, E. L., J. D. Pearson, and L. L. Slakey. 1986. The hydrolysis of extracellular adenine nucleotides by cultured endothelial cells from pig aorta. Feed-forward inhibition of adenosine production at the cell surface. *J. Biol. Chem.* 261:15496-15507.
15. Holmsen, H., C. A. Setkowsky, B. Lages, H. J. Day, H. J. Weiss, and M. C. Scrutton. 1975. Content and thrombin-induced release of acid hydrolases in gel-filtered platelets from patients with storage pool disease. *Blood.* 46:131-142.
16. Lieberman, G. E., G. P. Lewis, and T. J. Peters. 1977. A membrane-bound enzyme in rabbit aorta capable of inhibiting adenosine-diphosphate-induced platelet aggregation. *Lancet.* ii:330-332.
17. Furchgott, R. F., and J. V. Zawadzki. 1980. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature (Lond.).* 288:373-376.
18. Palmer, R. M. J., A. G. Ferrige, and S. Moncada. 1987. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature (Lond.).* 327:524-526.
19. Ignarro, L. J. 1990. Biosynthesis and metabolism of endothelium-derived nitric oxide. *Annu. Rev. Pharmacol. Toxicol.* 30:535-560.
20. Brockman, M. J., A. M. Eiroa, and A. J. Marcus. 1991. Inhibition of human platelet reactivity by endothelium-derived relaxing factor from human umbilical vein endothelial cells in suspension. Blockade of aggregation and secretion by an aspirin-insensitive mechanism. *Blood.* 78:1033-1040.
21. Glasgow, J. G., R. Schade, and F. A. Pitlick. 1978. Evidence that ADF hydrolysis by human cells is related to thrombogenic potential. *Thromb. Res.* 13:255-266.